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Are the Hydrogen Bonds of RNA (A·U) Stronger Than those of DNA (A·T)? A Quantum Mechanics Study

Alberto Pérez,[a] Jiri Sponer,[b, c] Petr Jurecka,[b, c] Pavel Hobza,[c] F. Javier Luque,*[d] and Modesto Orozco*[a, e]

Abstract: The intrinsic stability of Watson–Crick d(A·T) and r(A·U) hydrogen bonds was analyzed by employing a variety of quantum-mechanical techniques, such as energy calculations, determination of reactivity indexes, and analysis of electron density topology. The analyses were performed not only for equilibrium gas-phase geometries, but also on hundreds of conformations derived from molecular dynamics (MD) and database analysis. None of our results support the idea that r(A·U) hydrogen bonds are intrinsically more stable than those of d(A·T). Instead, our data are in accordance with the traditional view that the greater stability of RNA relative to DNA is attributable to a variety of effects (e.g., stacking, sugar puckering, solvation) rather than to a significant difference in the hydrogen bonding of DNA and RNA base pairs.

Keywords: density functional calculations · DNA · hydrogen bonds · quantum mechanics · RNA

Introduction

It is known that for equivalent complementary sequences, the RNA duplex is more stable than the DNA duplex under most laboratory and physiological conditions.[1] Traditionally, this has been explained by a variety of factors, including differential stacking, sugar puckering, and solvation.[1] This generally accepted view has been recently challenged by the results of various experimental studies suggesting that the hydrogen bonds (HBs) in A·U are intrinsically more stable than those of A·T. For example, Chattopadhyaya and coworkers[2] found the pK_a values of the donors and acceptors of r(A·U) pairs to be more similar than those of d(A·T) pairs. This was interpreted as evidence for the greater intrinsic strength of the A·U HBs. In addition, from measurements of C2 chemical shifts and deuterium isotope effects in adenine, Vakonakis and LiWang[3] concluded that the r(A·U) HBs are intrinsically stronger (around 0.4 kcal mol^{-1}/step) than those of d(A·T). These two experimental studies challenge our current understanding of the stability of nucleic acids, because the small differences in energy between d(A·T) and r(A·U) pairing, when propagated along long double helices, result in a major difference in the stability of DNA and RNA duplexes.

Theoretically, the suggested greater stability of A·U pairs is counterintuitive, if the large chemical and structural similarity between thymine and uracil is considered. Furthermore, the results of gas-phase quantum-mechanical (QM) studies have never suggested an enhanced intrinsic stability of HBs in A·U.[4] For example, Bickelhaupt and co-workers[5] recently reported a marginal difference (0.08 kcal mol^{-1} from BP86/TZ2P calculations) between A·T and A·U pairs, and provided convincing arguments that the results of NMR experiments do not directly reflect the strength of HBs. This raises doubts concerning the existence of a systematic and

[a] A. Pérez, Prof. M. Orozco
Molecular Modeling Unit, IRBB-PCB, Josep Samitier 1–5
Barcelona 08028 (Spain)
Fax: (+34) 934037157
E-mail: modesto@mmb.pcb.ub.es

[b] Prof. J. Sponer, P. Jurecka
Institute of Biophysics, Academy of Sciences of the Czech Republic
Kralovopolska 135, 61265 Brno (Czech Republic)

[c] Prof. J. Sponer, P. Jurecka, Prof. P. Hobza
Institute of Organic Chemistry and Biochemistry
Academy of Sciences of the Czech Republic
Flemingovo Nam 2, Prague (Czech Republic)

[d] Prof. F. J. Luque
Departament de Fisicoquímica, Facultat de Farmàcia
Universitat de Barcelona, Avgda Diagonal 643, Barcelona 08028 (Spain)
Fax: (+34) 934-037-157
E-mail: fjluque@ub.edu

[e] Prof. M. Orozco
Departament de Bioquímica i Biología Molecular
Facultat de Química, Universitat de Barcelona
Martí i Franquès1, Barcelona 08028 (Spain)
Fax: (+34) 934037157

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biologically meaningful difference in the stability afforded by HBs in A·T and A·U. Here, we have examined this problem by using QM computations, and found no evidence to support the idea that A·U HBs are stronger than A·T HBs. Instead, our results support the classical view of the relative stability of DNA and RNA, which relies on a subtle balance between stacking, solvation, and intrastrand energy.

Experimental Section

Gas-phase optimum geometries: We first analyzed the intrinsic stability of A·T and A·U in the gas phase. To achieve this, the dimers were fully optimized at the RI-MP2/cc-pVTZ level, and the interaction energies were determined at the MP2-complete-basis-set level (extrapolated from MP2/aug-cc-pVDZ and MP2/aug-cc-pVTZ calculations[6]) with CCSD(T) corrections computed with the 6–31G*(0.25) basis set[6] (this level is referred in the text as CCSD(T)/CBS). Energy corrections arising from geometry distortion and basis-set superposition errors were corrected by using standard procedures.[6] Calculations were also performed at the B3LYP/6–31G(d,p) level (using both RI-MP2 and DFT geometries), which allowed us to verify the quality of simple DFT calculations for the evaluation of the relative stabilities of hydrogen-bonded complexes of nucleic acid bases. Bader's[7] and GMIPs[8] analyses were used to examine further the stability of individual HBs in A·T and A·U base pairs. The CCSD(T)/CBS level is the state-of-the-art for quantum-mechanical calculations of interaction energies, and yields results that reproduce very accurately experimental data for a variety of hydrogen-bonded systems.[9]

RNA/DNA adapted geometries: Because calculations on the optimum gas-phase geometries might not properly reflect the situation in the DNA/RNA duplex, we extended our QM study to five A·T and A·U pairs derived from crystal geometries. For this limited set of structures (taken from protein data bank (PDB) entries showing very typical, classical interaction energies: bd0002, bd0004, bd0007, bd0031, bdj052, ar0006, ar0008, ar0022, arn0035, and url029), interbase geometries were fixed at crystal values, and intrabase geometries were optimized at the DFT level. Interaction energies were then computed (with BSSE correction) at CCSD(T)/CBS levels (distortion terms were determined at the DFT level). For comparison, these calculations were repeated at the B3LYP/6–31G(d) level and also classically by using the parm98 force-field.[10]

As described below, these calculations allowed us to verify the quality of the B3LYP/6–31G(d,p) results. Accordingly, this level of calculation was used to analyze the A·T and A·U pairing in a large subset[11] of B-DNA and A-RNA duplex crystal structures collected from the PDB that were at least 8-mer long and contained no drug/protein overhanging, mismatching, or modified nucleobases (a complete list of structures is available from the authors upon request). This subset was further filtered to remove all the G·C steps, the terminal A·T/U dimers, and any unusual pairs that showed unfavorable (positive) interaction energies. Backbones were replaced by a proton, and all of the intrabase geometries of the nucleobases (147 A·T, 30 A·U) were optimized at the B3LYP/6–31G(d,p) level, keeping the X-ray interbase geometry fixed. Finally, interaction energies corrected by using BSSE and geometry distortion terms were computed and averaged (individual data are available upon request). As before, Bader's analysis was performed at the B3LYP/6–31G(d,p) level for all of the optimized dimers.

Additionally, classical calculations were performed by taking 1000 geometries of d(A·T) and r(A·U) dimers, randomly selected from 10 ns molecular dynamics (MD) trajectory in aqueous solution.[12] These simple calculations helped us to detect possible bias in the results, by the use of crystal geometries instead of solution structures.

The effect of specific water molecules: The effect of tightly bound water molecules in modulating the strength of A·T/A·U HBs in DNA/RNA was explored by defining consensus hydration sites for DNA and RNA. These were obtained by inspecting 10 ns MD simulations of DNA and RNA duplexes of the same sequence.[13] This analysis showed the presence of two regions of very high water density for RNA (located in both major and minor grooves) and one for DNA (in the minor groove). TIP3P[13] water molecules were placed in the center of these regions and oriented for the different crystal or MD geometries of the A·T and A·U pairs by using parm98. The wave functions of the A·T/U pairs were then obtained by considering the perturbing effect of the atomic charges of TIP3P[13] water molecules in B3LYP/6–31G(d,p) calculations. Bader's analysis was also performed on the resulting electron density obtained for these microsolvated systems. This type of calculation allowed us to investigate specifically the polarizing effect of water on the strength of the hydrogen bond.

Quantum-mechanical calculations were performed by using Turbomole,[14] Gaussian-03,[15] PROAIMS,[16] and MOPETE[17] software packages. The remaining calculations/structural manipulations were performed by using home software.

Results and Discussion

The best results of the ab initio calculations (complete basis set/MP2 with CCSD(T) correction, hereafter referred to as CCSD(T)/CBS) suggest a marginal difference (only 0.1 kcal mol^{-1}) in the stability of A·T and A·U base pairs if the geometry is fully optimized in the gas phase (see Table 1). Interestingly, B3LYP/6–31G(d,p) calculations (performed by using either RI-MP2 or DFT geometries) decrease the interaction energy (in absolute terms) by 2.7 kcal mol^{-1}, but the energy difference between A·T and A·U remains unaltered. This supports the quality of the DFT calculations (see above).

Table 1. Interaction energies (kcal mol^{-1}) computed at the CBS/CCSD(T) and B3LYP/6–31G(d,p) levels for A·T and A·U dimers by using geometries optimized at the RI-MP2/cc-pVTZ (MP2) and B3LYP/6–31G(d,p) (DFT) levels (see text).

Dimer	ΔE(CCSD(T)/CBS)	ΔE(DFT)
A·T(MP2)	−15.0	−12.0
A·T(DFT)	–	−12.3
A·U(MP2)	−15.1	−12.1
A·U(DFT)	–	−12.3

As described in the Experimental Section, dimerization energy involves many different elemental interactions other than the stabilization due purely to hydrogen bonding between donor and acceptor atoms. Fortunately, topological analysis of electron density by using Bader's analysis provides a simple method to isolate the contribution of hydrogen bonds alone. Stronger HBs correspond to higher electron densities at the corresponding bond critical points (BCPs), and typically to larger values of the Laplacian.[7] The data in Table 2, obtained from either MP2/6–31G(d,p) or B3LYP/6–31G(d,p) electron densities, suggest that the N1−N3 hydrogen bond seems to be slightly stronger than the N6−O4 bond for both A·T and A·U pairs (the use of MP2 or DFT geometries does not alter the results). The differences in the properties of the BCPs between A·T and A·U are very small and compensate each other. For example, the N6−O4 HB seems to be slightly stronger for A·T

Table 2. Electron density ($\rho(r)$; au) and its Laplacian ($\nabla^2\rho(r)$; au) at the bond critical points connecting HB donor and acceptor atoms obtained from Bader's analysis by using B3LYP/6–31G(d,p) (non-italic) or MP2/6–31G(d,p) (italic) wave functions, and either RI-MP2/cc-pVTZ (MP2) or B3LYP/6–31G(d,p) (DFT) levels. GMIPp (HF/6–31G(d,p) in kcal mol^{-1}) was computed for T and U donor (N3) and acceptor (O4) atoms that were connected with the corresponding acceptor/donor groups in the paired A (see text).

Dimer	H-bond	$\rho(r)\times10^{-2}$	$\nabla^2(R)\times10^{-2}$	GMIPp
A·T(MP2)	N6–O4	3.3/*3.2*	9.3/*9.5*	−7.9
	N1–N3	4.4/*4.4*	9.8/*10.1*	−4.9
A·T(DFT)	N6–O4	2.8	7.6	−7.9
	N1–N3	4.3	9.6	−4.9
A·U(MP2)	N6–O4	2.9/*4.7*	8.3/*10.2*	−7.8
	N1–N3	2.9/*4.6*	8.6/*10.5*	−5.0
A·U(DFT)	N6–O4	2.8	7.5	−7.8
	N1–N3	4.4	9.6	−5.0

than for A·U, whereas the reverse is true for the N1–N3 bond. The sums of the electron densities at the N1–N3 and N6–O4 BCPs for the A·T and A·U pairs are equal, suggesting that no difference exists (for optimum gas-phase geometries) between the strength of HBs in A·T and A·U pairs. This conclusion is supported by the results of GMIPp analysis, which demonstrates that no difference exists in the hydrogen-bond donor/acceptor propensities of T and U (see Table 2).

An inevitable criticism of the results presented above is that they correspond to an ideal situation in the gas phase and not to that found in DNA and RNA polymers, in which geometrical restrictions can bias the A·T/A·U interactions and favour one interaction over the other. Calculations for ten (5+5) crystal structures of A·T and A·U dimers in real DNA and RNA orientations (see Experimental Section for details) confirms that subtle geometrical changes required for the adaptation of both A·T and A·U to the structure of DNA/RNA lead to non-negligible changes in the stability of the dimers (see Table 3). However, in relative terms, these

Table 3. Average interaction energies (CCSD(T)/CBS, B3LYP/6–31G(d,p), and parm98 in kcal mol^{-1}) and standard errors (also in kcal mol^{-1}) for five A·T and five A·U dimers in typical DNA and RNA conformations (see text). Individual values are available from the authors upon request.

Dimer	CCSD(T)/CBS	B3LYP/6–31G(d,p)	Parm98
A·T	-14.4 ± 0.3[a]	-11.6 ± 0.2	-13.4 ± 0.2
A·U	-13.9 ± 0.3[a]	-11.2 ± 0.3	-12.8 ± 0.3

[a] Geometries and distortion energies computed by performing DFT calculations.

changes are small and actually suggest that A·T pairs are more stable than A·U pairs, which is just the reverse to the suggestion derived from NMR spectroscopy experiments (see Introduction). Interestingly, as expected from the results in Table 1, B3LYP/6–31G(d,p) results reproduce very accurately the relative stability of A·T to A·U (note the shift of 2.7 kcal mol^{-1} between CCSD(T)/CBS and DFT interaction energies). Surprisingly, force-field results also reflect the relative difference between A·T and A·U pairs, which supports the suitability of force-field-based methods to analyze DNA and RNA structures.

The ability of DFT calculations to reproduce correctly interaction energies (Tables 1 and 3) and electron density topology (Table 2), as well as the computational efficiency of this method, makes it an ideal choice for the large-scale analysis of d(A·T) and r(A·U) dimers in the PDB (see Experimental Section). Interaction energies (including BSSE and distortion corrections) computed for all of the geometries (see Table 4, individual values are available upon request), show that upon moving from optimum gas-phase geometries to those found in the crystal, there is a non-negligible loss of stability in the A·T and A·U dimers (around 2 kcal mol^{-1} from the data in Tables 1 and 4). This cannot be justified completely by errors in refinement of the structures, and reflects how polymer restrictions avoid the formation of an optimum hydrogen-bonding arrangement in DNA and RNA duplexes. In addition, the interaction energies in Table 4 do not support the idea that A·U (RNA) pairs are more stable than A·T (DNA) pairs. In fact, any differences that do exist support the opposite situation. The results do not change if outliers (pairs showing anomalously poor interaction energies, i.e., with energies larger than three standard deviations from the average) are eliminated from the study. Furthermore, classical (parm98) analysis performed on 1000 configurations of d(A·T) and r(A·U) collected from structures sampled along MD simulations of duplex DNA and RNA revealed the same trends, suggesting that possible packing effects in the crystal do not bias the analysis (Figure 1).

Table 4. Average interaction energies and standard errors (in kcal mol^{-1}) computed at the DFT and classical (parm98) levels for crystal and MD A·T and A·U dimers (see text for details). Each cell contains values for the entire crystal data base (top), those obtained eliminating outlier pairs (those with interaction energies that deviate by more than three times the standard deviation from the average value (middle)), and values obtained from the ensemble of structures collected from MD (bottom).

Dimer	ΔE(DFT)	ΔE(Parm98)
A·T	-10.3 ± 0.2	-11.5 ± 0.2
	-10.5 ± 0.2	-11.6 ± 0.2
	–	-11.2 ± 0.0
A·U	-10.1 ± 0.4	-11.1 ± 0.4
	-10.1 ± 0.4	-11.1 ± 0.4
	–	-10.8 ± 0.0

Results of electron density topology analysis (Table 5) performed for all the dimers (individual values are available upon request) show a consistent reduction in the strength of HBs upon moving from ideal gas-phase to crystal geometries (see Tables 2 and 5), which is in accordance with the behavior of interaction energies. These analyses failed to reveal any difference in the strength of either N6–O4 or N1–N3 HBs in A·T and A·U pairs when the crystal geometries of both pairs in DNA and RNA are considered. A test

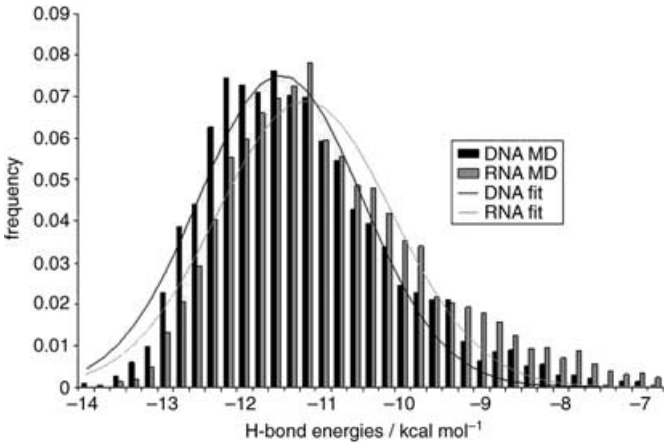

Figure 1. Representation of the distribution of the stability energy of d(A·T) and r(A·U) pairs obtained from MD simulations of DNA and RNA duplexes. Distribution plots are normalized to 1 and energy values are in kcal mol^{-1}. Lines correspond to the best fitted Gaussians.

Table 5. Average electron density ($\rho(r)$; au) and its Laplacian ($\nabla^2\rho(r)$; au) and the corresponding standard errors at the bond critical points connecting HB donor and acceptor atoms, obtained from Bader's analysis (see text for details) for d(A·T) and r(A·U) dimers. Non-italic values were obtained by considering the entire set of dimers, italic values were obtained after elimination of outliers (dimers with interaction energies that deviate by more than three times the standard deviation from the average value).

Dimer	H-bond	$\rho(r)\times10^{-2}$	$\nabla^2(R)\times10^{-2}$
A·T	N6–O4	$2.5\pm0.1/\mathit{2.5}\pm0.1$	$7.1\pm0.3/\mathit{7.1}\pm0.3$
	N1–N3	$4.7\pm0.1/\mathit{4.8}\pm0.1$	$9.9\pm0.2/\mathit{9.9}\pm0.2$
A·U	N6–O4	$2.7\pm0.2/\mathit{2.7}\pm0.2$	$7.6\pm0.5/\mathit{7.6}\pm0.5$
	N1–N3	$4.6\pm0.2/\mathit{4.6}\pm0.2$	$9.6\pm0.3/\mathit{9.6}\pm0.3$

calculation performed by using the MD-average conformation of d(A·T) and r(A·U) pairs in dodecamers (data not shown) confirms the conclusions derived here from crystal structures.

Our final concern was whether or not the different solvation patterns in DNA and RNA duplexes could polarize A·T and A·U dimers differently, and thus increase the strength of HBs in the A·U dimers. To analyze this, we microsolvated all of the crystal d(A·T) and r(A·U) by using average solvation contours detected from extended MD simulations of DNA and RNA duplexes (see Experimental Section). Results of Bader's analysis on these microsolvated clusters (see Table 6) showed that the impact of structural water molecules on the strength of A·T and A·U HBs is very small and does not alter the conclusions derived from the analysis of unsolvated crystal dimers.

In summary, our analyses of the d(A·T) and r(A·U) dimers, performed by applying state-of-the-art quantum theory, and by considering not only optimum gas-phase geometries, but also crystal conformations found in DNA and RNA duplexes, failed to provide any convincing evidence that: 1) A·U pairs are intrinsically more stable than A·T pairs, and 2) A·U HBs are stronger than A·T HBs. These findings confirm results from Swart et al.[5] and do not support suggestions by Acharya et al.[2] and Vakonakis and LiWang.[3] Therefore, our data suggest that differences between the stability of RNA duplexes containing A·U pairs and DNA duplexes containing A·T dimers are due to a variety of effects, including bulk solvation, different sugar puckering, changes in intra- and intermolecular stacking, and other subtle contributions related to the global conformation of DNA and RNA duplexes. Our findings support the classical explanation of the relative stability of DNA and RNA duplexes, rather than the alternative view that systematic differences in the intrinsic strength of A·T and A·U HBs are a major determinant of the greater stability of RNA duplexes containing A·U pairs relative to DNA duplexes containing A·T dimers.

Table 6. Average electron density ($\rho(r)$; au) and its Laplacian ($\nabla^2\rho(r)$; au) and the corresponding standard errors at the bond critical points connecting HB donor and acceptor atoms obtained from Bader's analysis (see text for details) for d(A·T) and r(A·U) microsolvated dimers. Non-italic values were obtained by considering the entire set of dimers, italic values were obtained after elimination of outliers (dimers with interaction energies that deviate by more than three times the standard deviation from the average value).

Dimer	H-bond	$\rho(r)\times10^{-2}$	$\nabla^2(R)\times10^{-2}$
A·T	N6–O4	$2.5\pm0.1/\mathit{2.5}\pm0.1$	$7.7\pm0.3/\mathit{7.7}\pm0.3$
	N1–N3	$4.7\pm0.1/\mathit{4.7}\pm0.1$	$9.8\pm0.2/\mathit{9.8}\pm0.2$
A·U	N6–O4	$2.6\pm0.2/\mathit{2.6}\pm0.2$	$7.7\pm0.5/\mathit{7.7}\pm0.5$
	N1–N3	$4.5\pm0.2/\mathit{4.5}\pm0.2$	$9.9\pm0.3/\mathit{9.9}\pm0.3$

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